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TUMORIGENESIS OF DIESEL EXHAUST, GASOLINE EXHAUST, AND RELATED EMISSION EXTRACTS ON SENCAR MOUSE SKIN *

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DISCLAIMER

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TUMORIGENESIS OF DIESEL EXHAUST, GASOLINE EXHAUST, AND RELATED EMISSION EXTRACTS ON SENCAR MOUSE SKIN[1,2]

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INTRODUCTION

Recent advances in the field of particulate emissions have brought to light several facts concerning their health effects. (1) Most emission sources produce respirable particles which have organic substances associated with the inert matrix (Waters et al., 1979). (2) These organic substances result from pyrosynthetic reactions at or near the combustion source and photosynthetic and oxidative processes which occur subsequent to their initial formation (Crittenden and Long, 1976). Many of these organic substances are extractable by biological systems from the inert matrix (McCormick et al., 1980). (3) Some of these materials contain known carcinogens and mutagens (Waters et al., 1979). Previous work by Kotin and coworkers and Mittler and Nicholson indicated

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[2]A preliminary account of this paper was presented at the International Symposium on the Health Effects of Diesel Exhaust (Slaga et al., 1980b).

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conflicting results on the mouse skin tumorigenicity of diesel exhaust components (Kotin et al., 1966; and Mittler and Nicholson, 1957). Similar studies with gasoline exhaust revealed a positive tumorigenic response upon multiple application on mouse skin of condensates and extracts (Kotin et al., 1964; Mittler and Nicholson, 1957; Hoffmann and Wynder, 1963; Hoffmann et al., 1965). This study was performed to examine the tumorigenicity of diesel exhaust particulate emissions using a sensitive mouse skin tumorigenesis model (SENCAR) and to compare the tumorigenic potency of particulate emissions from diesel, gasoline, and related emission sources.

The SENCAR mouse is a relatively new stock of carcinogen-sensitive animals which up to this time has not been used extensively in bioassay programs. A description of the SENCAR system and of mouse skin tumorigenesis in general follows in order to understand the strengths and weaknesses of this short-term in vivo carcinogenesis bioassay.

The SENCAR mouse is a mouse stock selected for its increased sensitivity to two-stage carcinogenesis using 7,12-dimethylbenz(a)anthracene (DMBA) as the initiator and 12-0-tetradecanoylphorbol-13-acetate (TPA) as the promotor. This system, however, is also more sensitive to other polycyclic aromatic hydrocarbons such as benzo(a)pyrene (Slaga et al., 1980a). In addition to its well documented ability to detect polycyclic aromatic hydrocarbons (Slaga et al., 1978b), the mouse skin tumorigenesis bioassay system has identified many chemicals other than polycyclic aromatic hydrocarbons as potential carcinogens (Table 1). These chemicals represent a wide variety of structural classes including: aldehyde; carbamate; epoxide; haloalkyether; haloaromatic, haloalkylketone, acid; hydroxylamine, lactone; nitrosamide; sulfonate; sultone; and urea. This list of 32 chemicals includes such well known chemical carcinogens as aflatoxin B1; bis(chloromethyl)ether; chloromethyl methyl ether; urethane; N-acetoxy-2-acetamidofluorene; β-propiolactone; N-methyl-N'-nitro-N-nitrosoguanidine; 1,3-propanesultone; N-nitrosomethyl urea; triethylenemelamine; and 4-nitroquinoline-N-oxide. In addition to the mouse skin tumorigenesis bioassay's response to chemicals other than polycyclic aromatic hydrocarbons, this system can also detect chemicals which give tumors in the respiratory tract of animals (Table 2). Of 11 known animal respiratory carcinogens, the mouse skin tumorigenesis system has to this date detected polycyclic aromatic hydrocarbons, quinolines, and carbamates. Of the 11 highly suspect occupational respiratory carcinogens, the mouse skin tumorigenesis system has to this date detected chloromethyl ethers and coke oven emissions. This indicates that the mouse skin tumorigenesis bioassay has a broad spectrum capability for detecting agents that are both dermal as well as nondermal carcinogens.

Table 1. Chemicals Other Than Polycyclic Aromatic Hydrocarbons Detected by Mouse Skin Bioassay

Class	Chemical	Reference
Aldehyde:	Malonaldehyde	Shamberger et al. (1974)
Carbamate:	Urethane	Salaman and Roe (1953)
	Vinyl carbamate	Slaga et al. (1973)
	Ethyl N-phenylcarbamate	Dahl et al. (1973)
		Dahl et al. (1980)
		Roe and Salaman (1955)
Epoxide, Diepoxide:	Glycidaldehyde	Shamberger et al. (1974)
		Van Duuren et al. (1965)
	1,2,3,4-Diepoxybutane	Van Duuren et al. (1965)
	1,2,4,5-Diepoxypentane	Van Duuren et al. (1965)
	1,2,6,7-Diepoxyheptane	Van Duuren et al. (1965)
	Chloroethylene oxide	Zajdela et al. (1980)
Haloalkylether:	Bis(chloromethyl)ether	Van Duuren et al. (1969)
		Zajdela et al. (1980)
		Slaga et al. (1973)
	Chloromethyl methyl ether	Slaga et al. (1973)
		Van Duuren et al. (1969)
Haloaromatic:	2,3,4,5-Tetrachloronitrobenzene	Searle (1966)
	2,3,4,6-Tetrachloronitrobenzene	Searle (1966)
	2,3,5,6-Tetrachloronitrobenzene	Searle (1966)
	Pentachloronitrobenzene	Searle (1966)
Haloalkyl ketone, acid:	Chloroacetone	Searle (1966)
	3-Bromopropionic acid	Searle (1966)
Hydroxylamine:	N-Acetoxy-4-acetamidobiphenyl	Scribner and Slaga (1975)
	N-Acetoxy-2-acetamidofluorene	Scribner and Slaga (1975)
		Slaga et al. (1978b)
	N-Hydroxy-2-aminonaphthalene	Clayson and Garner (1976)
	N-Acetoxy-2-acetamidophenanthrene	Scribner and Slaga (1975)
	N-(4-Methoxy)benzoyloxypiperidine	Scribner and Slaga (1975)
	N-(4-Nitro)benzoyloxypiperidine	Scribner and Slaga (1975)
	N-Acetoxy-4-acetamidostilbene	Scribner and Slaga (1975)
Lactone:	β-Propiolactone	Roe and Salaman (1955)
		Slaga et al. (1973)
		Hennings and Boutwell (196
Multifunctional:	Triethylenemelamine	Roe and Salaman (1955)
	4-Nitroquinoline-N-oxide	Hennings and Boutwell (1969)
Natural Products:	Aflatoxin B1	Lindenfelser et al. (1974)
Nitrosamide:	N-Methyl-N'-nitro-N-nitrosoguanidine	Hennings et al. (1978)
		Fujii (1976)
Sulfonate:	Allyl methylsulfonate	Roe (1957)
Sultone:	1,3-Propanesultone	Slaga et al. (1973)
Urea:	N-Nitrosomethylurea	Graffi and Hoffman (1966)

Table 2. Response of Carcinogens in Humans, Animals, and Mouse Skin

	Occupational Respiratory Carcinogen[a]	Animal Respiratory Carcinogen[a]	Mouse Skin Tumorigen[b]
Arsenic	+		
Asbestos	+	+	
Beryllium	+	+	
Carbamates		+	+
Chloromethylethers	+	+	+
Chromium	+		
Coke oven	+		+
Isopropyl oil	+		
MOCA	+	+	
Mustard gas	+	+	
Nickel	+	+	
Nitrosamines		+	
Polycyclic aromatics		+	+
Quinolines		+	+
Vinyl chloride	+	+	

[a]Frank (1978).

[b]Slaga et al. (1978b), Slaga et al. (1980b), and Van Duuren (1976).

The two basic protocols that can be employed to detect chemical carcinogens in the mouse skin tumorigenesis assay are illustrated in Figure 1. Multiple application of the test agent for up to 60 weeks will mainly give rise to malignant carcinomas of the skin. This protocol, for complete carcinogens, is a test for agents exhibiting both tumor initiating and tumor promoting activities. The bioassay protocol for tumor initiators is a single application of test agent followed one week later by multiple applications of a potent tumor promoter. Tumor initiation is one step of the multi-step carcinogenic process and involves the conversion of a normal cell into a preneoplastic one. In the case of chemical carcinogens, it involves the interaction of chemicals or their activated forms with cellular DNA. These initiated cells remain dormant for periods up to one year or until they are stimulated to progress into hyperplastic or neoplastic lesions. This stimulation is called tumor

promotion and is accomplished by application of croton oil or its most active component, TPA. An initiated cell is, therefore, an irreversibly formed preneoplastic lesion which can be stimulated to express the transformed phenotype.

Relationships between tumor initiators and complete carcinogens have been previously described. A variety of structurally diverse chemicals found in Table 3 are both complete carcinogens and tumor initiators in mouse skin from CD-1 and the genetically related SENCAR mouse. There are, however, agents that appear to have only tumor initiating activities in mouse skin (Table 4). The quantitative relationship between chemicals which are complete carcinogens and tumor initiators is described in Table 5. There is excellent correlation between the relative potency of 12 chemicals which are both complete carcinogens and tumor initiators. The relationship between the production of papillomas and the production of carcinomas in the same animals treated with the skin tumor initiator DMBA or benzo(a)pyrene (B[a]P) is shown on Table 6. A good quantitative correlation is observed between the production of the number of papillomas after 15 weeks of scoring and the number of carcinomas after 50 weeks of scoring for animals treated with these two strong skin tumor initiators. This indicates that the number of papillomas per mouse at 15-20 weeks correlates well with the number of malignant carcinomas formed at 50 weeks.

Table 3. Compounds Which Are Complete Carcinogens and Tumor Initiators in CD-1 and SENCAR Mouse Skin[a]

7,12-Dimethylbenz(a)anthracene	β-Propiolactone
3-Methylcholanthrene	Bis chloromethyl ether
Benzo(a)pyrene	2-Hydroxybenzo(a)pyrene
7-Methylbenz(a)anthracene	Benzo(a)pyrene-7,8-oxide
Dibenz(a,h)anthracene	Benzo(a)pyrene-7,8-diol
5-Methylchrysene	7,12-Dimethylbenz(a)anthracene-3,4-diol

[a]Hecht et al. (1979), Slaga et al. (1978b), and Slaga et al. (1980b).

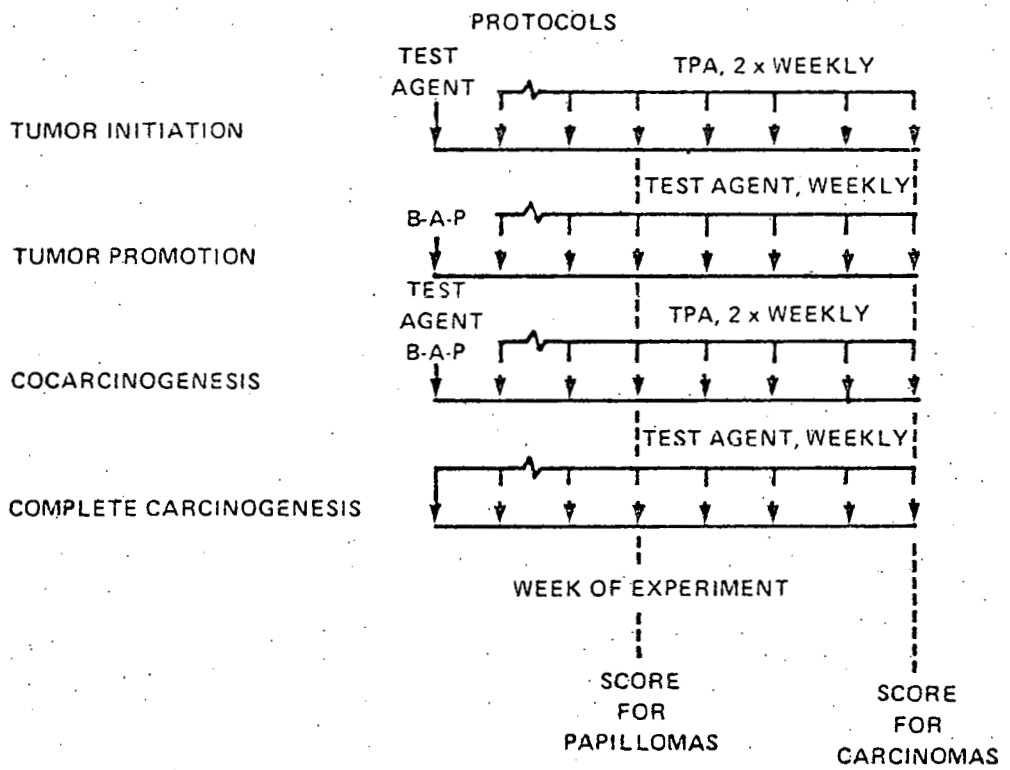

Fig. 1. Protocols for bioassay of test agents as tumor initiators, tumor promotion, cocarcinogens and complete carcinogens.

Table 4. Agents That Are Possibly Pure Tumor Initiators in Mouse Skin[a]

Benzo(a)pyrene-7,8-diol-9,10-epoxide
N-Methyl-N'-nitro-N-nitrosoguanidine
Benz(a)anthracene-3,4-diol-1,2-epoxide
Benz(a)anthracene
Dibenz(a,c)anthracene
Chrysene
Urethane
Triethylenemelamine

[a]Scribner (1973), Scribner and Slaga (1975), Slaga et al. (1973), Slaga, et al. (1978a), Slaga et al. (1979), and Van Duuren, (1976).

Table 5. Comparison of Complete Carcinogenesis and Tumor Initiation in Mouse Skin

Compound	Relative potency[a]	
	Complete carcinogenesis, carcinomas	Tumor initiation, papillomas
7,12-Dimethylbenz(a)anthracene	100	100
3-Methylcholanthrene	50	50
Benzo(a)pyrene	30	30
2-Hydroxybenzo(a)pyrene	30	30
7-Bromomethyl-12-methylbenz(a)anthracene	20	20
Benzo(a)pyrene-7,8-oxide	20	20
Dibenz(a,h)anthracene	20	20
Benz(a)anthracene	5±5	5
Dibenz(a,c)anthracene	0	3
Pyrene	0	0
Benzo(a)pyrene-4,5-oxide	0	0
Anthracene	0	0

[a]Relative potency was determined from dose-response data. 7,12-Dimethylbenz-(a)anthracene was given a maximum value of 100 (Slaga et al., 1980b).

Table 6. Dose Response Studies on the Ability of 7,12-Dimethylbenz(a)anthracene (DMBA) and Benzo(a)pyrene (B(a)P) to Initiate Skin Tumors in SENCAR Mice[a]

Initiator	Dose, nmoles	# of papillomas per mouse at 15 weeks[b]	% of mice with papillomas at 15 weeks	% of mice with carcinomas at 50 weeks
DMBA	100.0	22.0 (100)	100	100
DMBA	10.0	6.8 (32)	100	40
DMBA	1.0	3.2 (15)	93	22
DMBA	0.1	0.5 (2)	20	5
B(a)P	200.0	7.5 (100)	100	55 (100)
B(a)P	100.0	3.2 (43)	78	30 (55)
B(a)P	50.0	1.4 (19)	60	18 (33)

[a]Mice were treated one week after initiation with twice weekly application of 5 μg TPA.

[b]Values in parentheses represent % normalized to the highest dose tested of each agent (Slaga et al., 1980b).

MATERIALS AND METHODS

Sample Generation and Isolation

The details of sample generation and isolation have been reported elsewhere (Huisingh et al., 1980). Briefly, the mobile source samples consisted

Table 7. Mobile Source Sample Generation

Sample		Description	Fuel	Driving Cycle
Diesel:	Cat:	Caterpillar 3304	Diesel No. 2	Mode II[a]
	Nissan:	Nissan Datsun 220C	Diesel No. 2	HWFET[b]
	Olds	Oldsmobile 350	Diesel No. 2	HWFET
	VW Rab:	Volkswagon Turbocharged Rabbit	Diesel No. 2	HWFET
Gasoline:	Mustang:	1978 Mustang, II-302, V-8 Catalyst and EGR	Unleaded Gasoline	HWFET

[a]Mode II cycle was conducted at 2200 rpm steady state with an 85 lb load.

[b]Highway fuel economy cycle (HWFET) was a 10.24 mi cycle averaging 48 mph, and taking 12.75 min.

of particulate emissions from three diesel fueled engines (Table 7): a heavy-duty Caterpillar 3304 engine mounted on an engine dynamometer at 2200 rpm steady state with an 85 pound load, and a Datsun Nissan 220-C, Oldsmobile 350, and a 1978 Mustang II-302 V-8 catalyst engine (with emission controls using unleaded gasoline) mounted on a chassis dynamometer using a highway fuel economy cycle of 10.24 miles, an average speed of 48 mph, and a running time of 12.75 minutes. The Caterpillar, Datsun-Nissan, and Oldsmobile engines were fueled with the same batch of No. 2 diesel fuel. Particulate samples were collected using a dilution tunnel in which the hot exhaust was diluted, cooled, and filtered through Pallflex Teflon coated fiberglass filters.

The comparative sources employed were cigarette smoke condensate, coke oven and roofing tar emissions. Cigarette smoke condensate was obtained by condensing smoke from an 85 mm non-filter Kentucky reference cigarette 2R1. Condensate was collected in acetone and refrigerated Dry Ice-isopropanol bath. Cigarette smoke condensate acetone suspension was adjusted with appropriate amounts of acetone and water. Coke oven samples were collected from the top of a coke oven battery at Republic Steel, Gadston, Alabama, using the Battelle-Massive Volume Sampler. Due to local wind conditions, various types of aerosols were sampled; thus, an unknown but significant portion of the emission sample may have been from the urban environment. Roofing tar emission sample was collected using a conventional tar pot with external propane burner. Pitch based tar was heated to 360-380°F, and emissions were collected using a six foot stack extension and Teflon socks in a bag house.

The mobile source, coke oven, and roofing tar emissions were Soxhlet extracted with dichloromethane, the dichloromethane removed by evaporation under dry nitrogen, and the samples shipped in coded form in dry ice to Oak Ridge National Laboratories where the animal experiments were conducted. Table 8 describes the amount of organic material extracted from the particles with dichloromethane for each of the samples and the amount of B(a)P per milligram extract or per milligram particle in each sample. The B(a)P analysis was performed according to the method of Snook et al. (1976). Percent extractable of organic material from the particles varied from 8% of the Nissan sample to a maximum of 99% for the roofing tar sample. Since cigarette smoke condensate was not a particulate sample per se, the complete sample was used in the biological analysis. B(a)P in the extracts varies from less than one ng/mg extract for the cigarette sample to a high of 1173 ng/mg extract for the Nissan sample.

Animals

SENCAR mice, a mouse stock selected for its increased sensitivity to carcinogenesis as described by Boutwell were used in this study (Boutwell, 1964). These mice were derived from breeding Charles River CD-1 mice with male STS skin tumor sensitive mice which were originally derived from Rockland mice. Selection was performed by sensitivity to the DMBA-TPA two-stage system of tumorigenesis for eight generations. These mice were initially obtained from Dr. R. Boutwell, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin, and now are being raised at the Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Table 8. Benzo(a)pyrene Analysis[a]

Sample		Extractable (%)	ng B(a)P per mg extract	ng B(a)P per mg particle
Diesel:	Cat	27	2	0.5
	Nissan	8	1173	96.2
	Olds	17	2	0.4
	VW Rab	18	26	4.6
Gasoline:	Mustang	43	103	44.1
Comparative Sources:	Cigarette	-	<1	--
	Coke	7	478	31.5
	Roof Tar	>99	889	889

[a]B(a)P analysis was performed according to Snook et al. (1976).

Chemicals

TPA was obtained from Dr. P. Borchert, University of Minnesota, Minneapolis, MN, and B(a)P from Aldrich Chemical Co., Milwaukee, WI. All the agents were prepared under yellow light immediately before use and applied topically in 0.2 ml of spectral quality acetone.

Tumor Experiments

These studies employed 80 mice per treatment group (40 of each sex). All the mice were shaved with surgical clippers two days before the initial treatment, and only those mice in the resting phase of the hair cycle were used. Five dose levels were used for the tumor initiating activities of the various samples. B(a)P was used as the standard for the tumor initiation studies. One week after application, the tumor promotor TPA was administered twice weekly. All samples at all doses were applied as a single treatment except for the 10 mg dose which was administered in five daily doses of 2 mg. Skin tumor formation was recorded weekly and papillomas greater than 2 mm in diameter were included in the cumulative total if they persisted for one week or longer. Both the number of mice with tumors and the number of tumors per mouse were determined and recorded weekly. At random, papillomas and carcinomas were removed for histological verification.

RESULTS AND DISCUSSION

The organic extracts from particulate emissions described previously was applied to the backs of SENCAR mice according to the protocols cited in Materials and Methods. The production of benign papillomas on a weekly basis is described in Figure 2 for both the reference standard B(a)P and the Nissan sample. In both cases, after a 7-8 week latent period, the percent of animals bearing tumors rose dramatically between weeks 8-14 with a 95-100% tumor incidence observed in both of these dose groups. Mean number of papillomas per mouse began to rise from control between weeks 6-8 with a much slower increase than the number of animals with tumors and a plateau being reached at approximately weeks 22-25. In both cases, the numbers of papillomas per animal range from 5-6 per animal.

B(a)P exhibited a linear dose response between 2.52 and 100.92 μg (10-400 nmoles) in both male and female SENCAR mouse skin (Figure 3). The male animals seemed to be more sensitive than the female animals towards this carcinogen, although this sex difference was not evident in the complex mixture samples evaluated. The most active sample tested in this series was the coke oven extract. The response of this sample in both male and female animals was biphasic. An initial linear dose response was observed between 0.1 and 2 mg extract with animals carrying an average of 5-6 papillomas. The roofing tar extract and Nissan extract (Figure 4) also produced a large tumor response in both male and female animals.

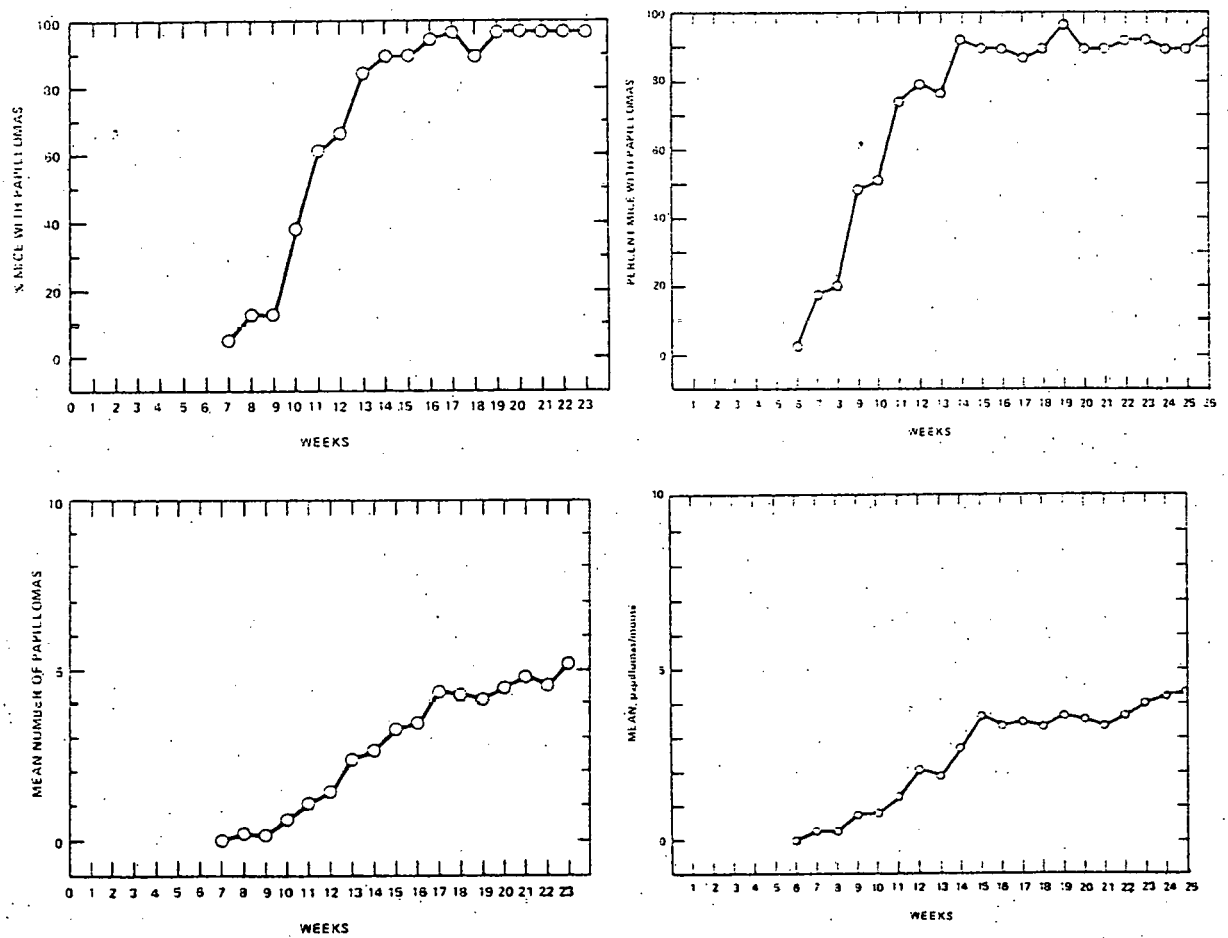

Fig. 2. SENCAR mouse skin tumor initiation. Male SENCAR mice (40) were initiated with either a single dose of benzo(a)pyrene (50.4 µg) or 5 daily treatments of Nissan extract (2 mg). Animals were then treated biweekly with TPA (2 µg) according to Materials and Methods. Numbers of mice with papillomas as a function of time after treatment. Upper left: B(a)P. Upper right: Nissan extract. Mean number of papillomas/mouse. Lower left: B(a)P. Lower right: Nissan extract.

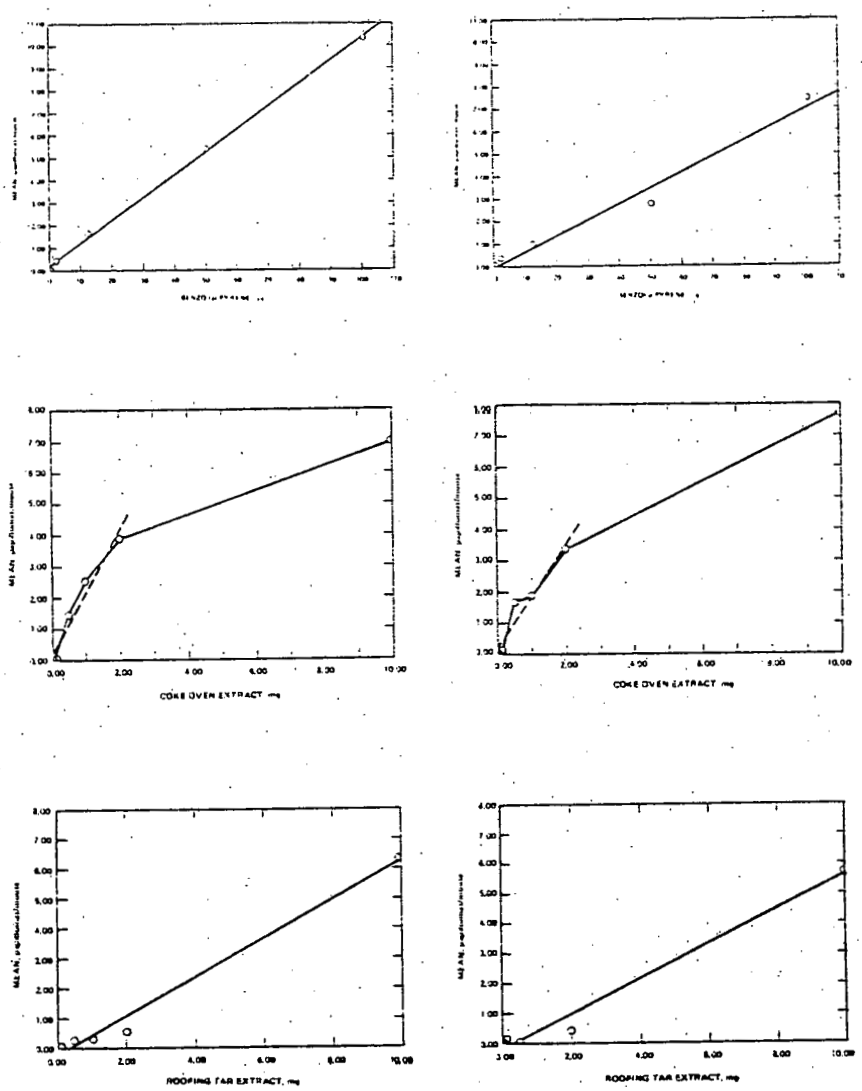

Fig. 3. SENCAR mouse skin tumor initiation. Dose response plots of mean papilloma formation/mouse (after background subtraction) and dose of test material. Animals were treated, housed, and scored according to procedures described in Materials and Methods. Forty animals were treated in each dose group (5 dose groups/sex/test agent) and the number of surviving animals at scoring is listed in parentheses after the sample name. Upper left: B(a)P (4 dose groups) - male (156). Upper right: B(a)P (4 dose groups) - female (156). Middle left: Coke oven extract - male (195). Middle right: Coke oven extract - female (197). Bottom left: Roofing tar extract - male (197). Bottom right: Roofing tar extract - female (196).

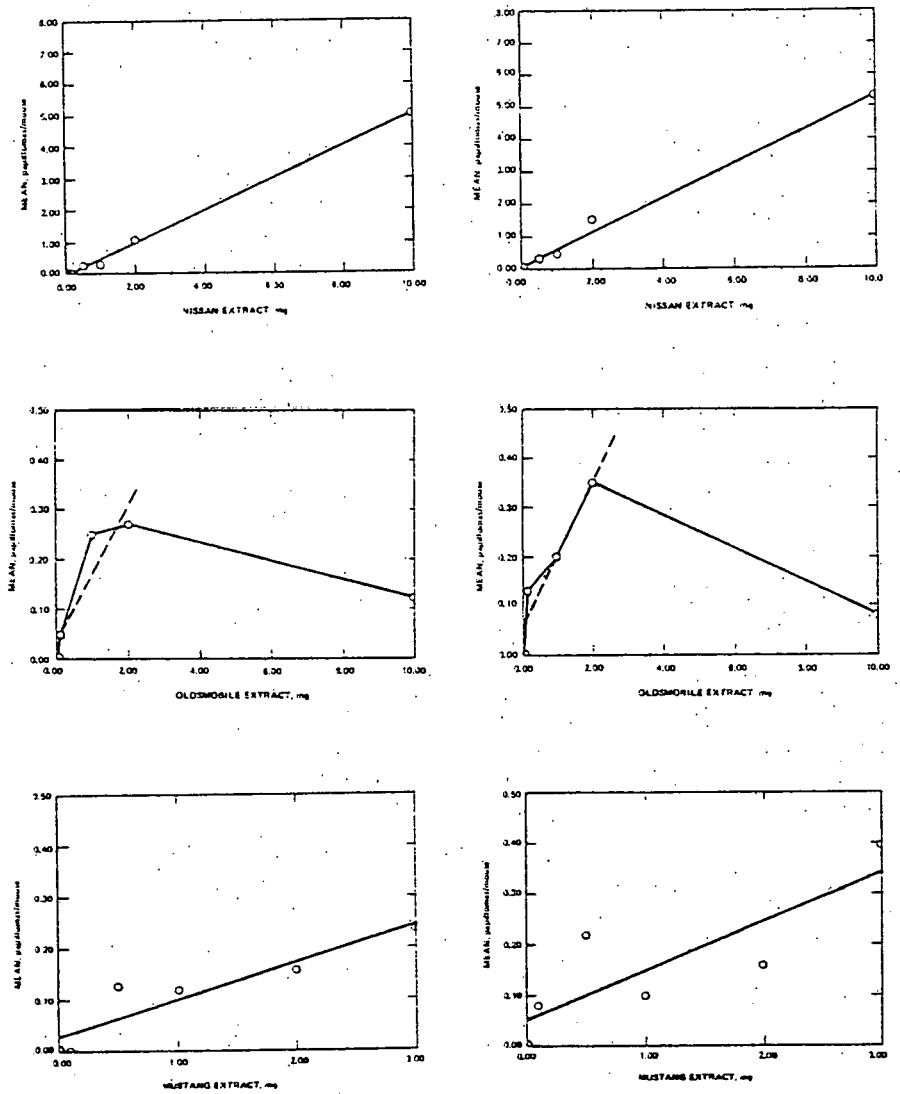

Fig. 4. SENCAR mouse skin tumor initiation dose response plots of mean papillomas/mouse (after background subtraction) and dose of test material. See Figure 3 legend for details. Upper left: Nissan extract - male (190). Upper right: Nissan extract - female (198). Middle left: Oldsmobile extract (4 dose groups) - male (156). Middle right: Oldsmobile extract (4 dose groups) - female (157). Lower left: Mustang extract - male (188). Lower right: Mustang extract - female (195).

The Oldsmobile sample exhibited a linear dose response up to 1 mg and a subsequent loss of activity at 10 mg. The magnitude of the Oldsmobile response was 10-fold lower than that found in the Nissan, coke oven, and roofing tar samples. The gasoline fueled Mustang II sample also produced a weak response in both male and female animals. The "goodness of fit" (R^2)to the linear regression analysis in the female animals was extremely low, 0.686, indicating a lack of linear dose response. The Caterpillar and cigarette smoke condensate produced two to three times the numbers of tumors as found in the controls (Figure 5). However, there was no observable dose response within the doses tested (0.1 to 10 mg). The lack of activity of the cigarette smoke condensate although disappointing was not unexpected. Cigarette smoke condensate when applied to female ICR Swiss mice twice weekly only produced tumors at relatively high dosages, (Gori et al., 1977; Wynder and Hoffmann, 1967). It was expected that the increased sensitivity of SENCAR mice to carcinogens would allow observation of tumors after treatment with 10 mg whole smoke condensate. This, however, was not the case. Cigarette smoke condensate is not an extract of isolated particulates but a suspension of organics, particles, and volatiles. It, therefore, has not been concentrated to the same extent as the other samples. The detectability limit of the SENCAR mouse skin tumorigenesis assay is above the doses and concentrations tested of the cigarette smoke condensate.

The formation of spontaneous tumors in animals treated with acetone and promoted twice weekly with TPA was 0.08 and 0.05 papillomas/mouse in male and female animals, respectively. This represented 7-8% of the animals with tumors at 22 weeks after initiation. Animals initiated with up to 100.92 µg of benzo(a)pyrene followed by promotion with acetone alone did not produce tumors.

A preliminary analysis of the results obtained was performed using a linear regression statistical analysis to produce potencies of papillomas/animal/mg agent. The results of these calculations are found in Table 10. The R^2 or "goodness of fit" of the data to the linear response was greater than 0.920 for eight of twelve of the test groups and greater than 0.84 for 11 out of 12. Potency values ranged from 0 to 101.3 papillomas/mouse/mg agent. The higher of these values were obtained from the B(a)P treatment groups and are an extrapolation from the microgram dose range where the data was obtained to the mg range. Obviously this number is theoretical and based on strict linearity throughout a 1000-fold dose range, an assumption not yet proven. Also, it is a physical impossibility to have 100 papillomas on the back of

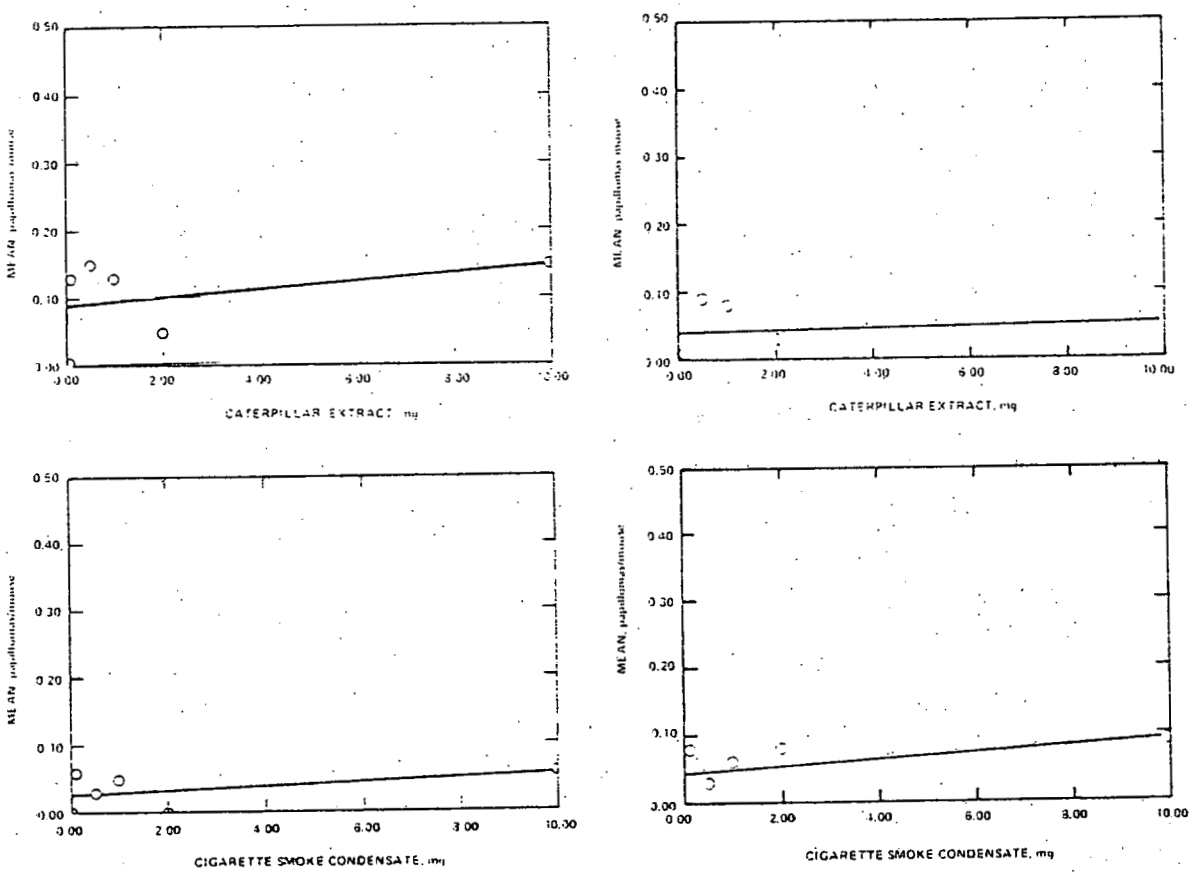

Fig. 5. SENCAR mouse skin tumor initiation dose response plots of mean papillomas/mouse (after background subtraction) and dose of test material. See Figure 3 legend for details. Upper left: Caterpillar extract - male (196). Upper right: Caterpillar extract - female (191). Lower right: Cigarette smoke condensate - male (187). Lower right: Cigarette smoke condensate - female (194).

a mouse. However, for comparative purposes, these values give a good approximation of the true values. A relative ranking of each of the test group to each other after normalizing to the Nissan sample is also found in Table 10.

The relative rankings of all the samples using the linear regression analysis indicate that B(a)P is greater in potency than coke oven which is greater than roofing tar and Nissan. These samples were greater than Oldsmobile or Mustang. These two samples were greater than cigarette smoke condensate and Caterpillar which were not statistically different than zero.

The results presented here confirm and expand the earlier observations by Kotin et al. on the tumorigenesis of diesel exhaust components (Kotin et al., 1966) and clearly indicate the tumorigenic potential of these materials. The results also indicate a range of response of diesel engines, presumably due to differences in engine technology.

Comparison of the tumor data in Table 10 with the B(a)P content per mg extract in Table 8 indicate a lack of correlation between the two parameters. This suggests that B(a)P and associated polycyclic aromatic hydrocarbons (PAH) are not reliable markers for tumorigenic activity in these complex mixtures and that other non-PAH chemicals in the mixtures make major contributions to their overall potency.

In conclusion, the SENCAR mouse skin tumorigenesis bioassay for tumor initiation is a quantitative short-term in vivo rodent carcinogenesis system which detects a variety of structurally diverse chemical carcinogens. This bioassay system has also shown its utility in evaluating complex environmental mixtures for tumorigenic potential. It gives excellent dose responses with both pure substances and complex mixtures and has shown utility for comparative potency analysis. Additional statistical models are being evaluated to analyze this data and the results will be reported elsewhere.

Table 10. SENCAR Mouse Skin Tumor Initiation: Sample Rankings[a]

	Papillomas/mouse/mg	R^2	Relative ranking
Benzo(a)pyrene	101.3 (M)	0.999	19980
	71.1 (F)	0.979	13365
Coke Oven	2.00 (M)	0.960	395
	1.65 (F)	0.922	310
Roofing Tar	0.640 (M)	0.975	126
	0.571 (F)	0.977	107
Nissan	0.532 (F)	0.991	100
	0.507 (M)	0.998	100
Olds	0.148 (F)	0.896	28
	0.135 (M)	0.844	27
Mustang	0.097 (F)	0.686	18
	0.073 (M)	0.842	14
Cigarette	0	-	0
Caterpillar	0	-	0

[a]A linear regression model was applied to the individual data points to obtain both slope potency and R^2. M and F refer to results from male and female animals, respectively.

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